RESEARCH PROPOSAL

Effects Of Thiamethoxam on Hepatic Function and Gene Expression in Male Albino Mice

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TABLE OF CONTENTS

S. No.	Contents	Page No
1.	**INTRODUCTION**	1
	1.1. Mode of Entry of pesticides	2
	1.2. Classification of pesticides	2
	1.2.1 Bio-pesticides	2
	1.2.2 Chemical pesticides	3
	1.2.3 Neonicotinoids and its types	3
	1.2.4 Thiamethoxam	5
2.	**REVIEW OF LITERATURE**	6
	2.1. Neonicotinoid Toxicity	6
	2.2. Hepatotoxicity	9
	2.3. Transcriptomics	10
3.	**OBJECTIVES**	12
4.	**METHODOLOGY**	13
	3.1. Experimental Model	13
	3.2. Animal experimentation and treatment plan	13
	3.3. Transcriptome Analysis	15
5.	**SIGNIFICANCE OF THE STUDY**	16
6.	**REFERENCES**	17

INTRODUCTION

India is mainly an agriculture based country, where agriculture plays a very important role in economic advancement and development. It has a significant contribution in providing food for continuously growing population and raw material for non-agriculture sector. The contribution of the agriculture sector is about 16% of the total GDP of India and 10% of the total export. During the 1930s and late 1960s, emergence of green revolution marked a drastic increase in the productivity globally due to the new advances like hybrid variety of seeds, use of chemical pesticides, herbicides and other agrochemicals. These synthetic chemicals prevented weeds, pests and diseases, as a result the crop yield increased with high nutrient levels.

Chemical fertilizers and synthetic pesticides proved to be very helpful for the crop production but they also had major side effects on insects which were useful to the plants and which do not come in the category of pests. They were also found to be the cause for environmental pollution causing bio magnification, soil pollution and water pollution.

The mode of entry of these pesticides can be by ingestion, inhalation or by contact as some water or fat soluble pesticides get absorbed by the skin very easily. The adverse effects also depend upon the amount of exposure of the individual to the pesticide (George *et al*., 2011). When exposure is for a short time period, its effects are known as acute effects and they can be determined by various superficial symptoms such as skin irritation, eye infections and oral infections. Effects from long term exposure or chronic effects are usually genetic defects, birth defects, fetal toxicity, cancerous tumor formation and various organ failures (Hou *et al*., 2017). Apart from these acute and chronic effects they also affect the immune system causing immuno-suppression and hypersensitivity reactions.

The use of insecticides and pesticides is drastically increasing as compared to the past. The insecticides which were first used were sulphur based. Also, mercury and arsenic were used for destroying harmful garden insects (Hvezdova *et al*., 2017). Tobacco was also utilized prominently as contact insecticide. Since chemical pesticides were not introduced initially, mainly plant and animal derived pesticides were used. The origin of synthetic pesticide was carried out during the World War II for production of biological weapons. During the war, DDT (Dichloro diethyl trichloro ethane), BHC (β-Benzene hexachloride), Aldrin, Dialdrin etc. came into existence (Ruus *et al*., 2010).

1.1. MODE OF ENTRY OF PESTICIDES

Different pesticides have different type of mode of entry and actions, some work by stomach poisoning which is ingested inside the pest's body by chewing, affecting the digestive tract and causing death by poisoning. The pesticides with this mode of action are known as stomach poison. Another mode is contact poisoning in which the pesticide enters via the epidermis (skin) upon contact primarily by penetrating the cuticle or outer body wall of the insect and cause death (Sparks and Nauen, 2014). Fumigation is another way by which the pesticides are taken up in gaseous form inside the body of the pest via respiratory system and causes its death. There is an indirect way known as Systemic Action, in which pesticide is taken up by a host plant and remains in it. Pests that feed on those plants are killed by the poison (Garcia *et al*., 2012).

1.2. CLASSIFICATION OF PESTICIDES

Pesticides are classified in different categories on the basis of their origin or the kind of pests they control. On the basis of origin they are classified into two classes (Fig. 1.1).

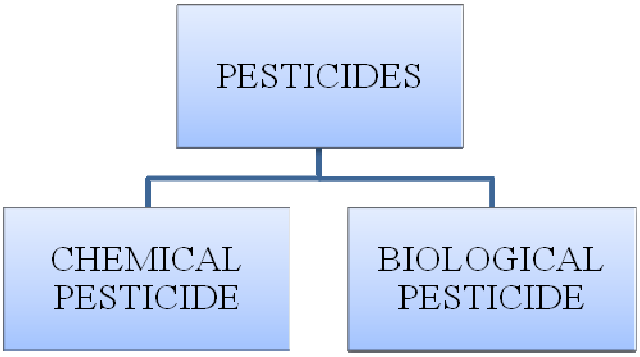


Figure 1.1: General classification of pesticides

1.2.1. BIO-PESTICIDES

Biopesticides are biological pesticides that are derive from animals, plants and bacteria or naturally occurring plant metabolites and enzymes that are used to eliminate pests which damage the agricultural produce. They are divided into three categories namely microbial pesticide, biochemical pesticide and PIPs (Plant Incorporated Protectants). Microorganisms such as bacteria, virus and fungi are used as pest repellents and they are target specific, one of the most popular examples is *Bacillus thuringenesis* or Bt. They work by producing a protein which binds to larval gut receptor (Fang-bin *et al*., 2017). Biochemical pesticides include pheromones, natural

plants or insect regulators, enzymes, bio-repellant or attractants and they are non-toxic to non-target organisms. PIPs are produced naturally by introducing the desired gene into plant by genetic engineering process.

1.2.2. CHEMICAL PESTICIDES

Chemical pesticides are mainly referred to synthetically produced compounds used for pest control in the agricultural sector. They are divided into five categories: Organophosphates, Carbamates, Organochlorines, Pyrethroids and Neonicotinoids. Both organophosphates and carbamate affect the nervous system by inhibition of enzyme acetylcholine esterase. In organophosphate, the bond formed is irreversible resulting in stocking up of acetylcholine which over stimulates the muscles, whereas in carbamate the bond formed is reversible (Siqueira *et al*., 2015). Chlorinated hydrocarbon or organochlorines mainly consist of endocrine disrupting agents affecting hormonal system by imitating the normal hormones therefore causing adverse results as they have a special quality of persisting and accumulating in organisms (Reiler *et al*., 2015). The fourth category is pyrethoid pesticide which is a neuro poison. It disrupts the endocrine system which ends up causing paralysis. One of the major advantages of this pesticide is that it passes through exoskeleton of the insect and kills the pests even by contact (Columé *et al*., 2001). The fifth class is a relative new class called neonicotinoid which are structurally and functionally similar to nicotine.

1.2.3. NEONICOTINOIDS AND ITS TYPES

The word “neonicotinoid” means “new nicotine like pesticide” and is structurally and functionally similar to nicotine. They are systemic pesticides and thus are easily absorbed by the plants. It is a new class of pesticide which is now being used extensively. The mode of action of neonicotinoid is very similar to that of natural nicotine as they are agonists on the polysynaptic nicotine acetylcholine receptors present in the CNS of the pests (Babelova *et Tal*., 2017).

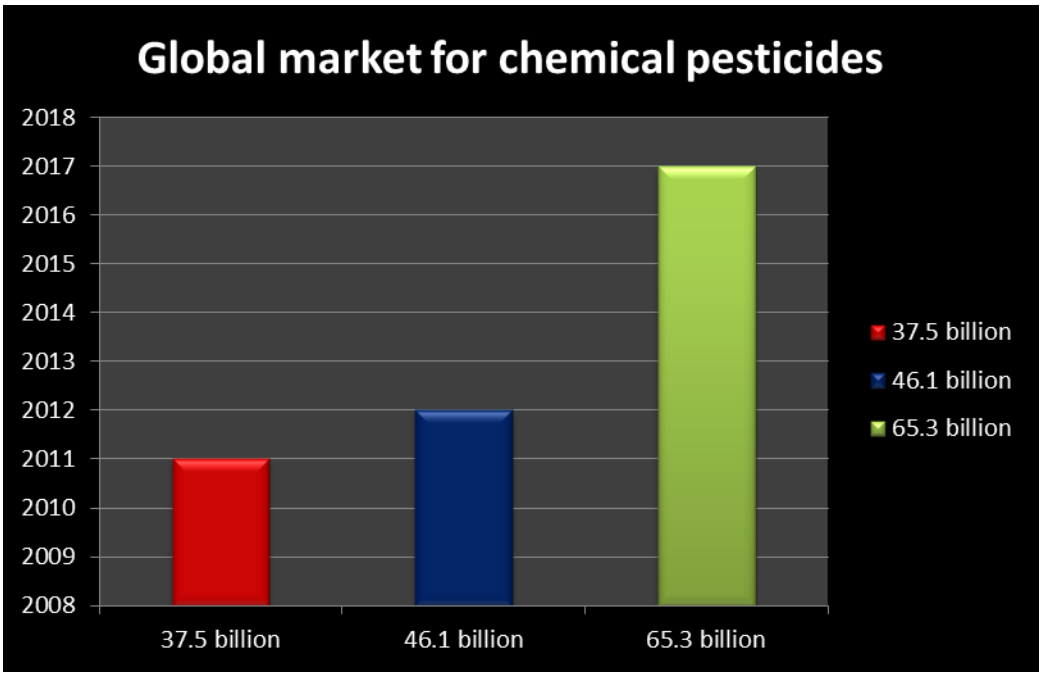


Figure 1.2 : The graph shows global distribution of chemical pesticides.

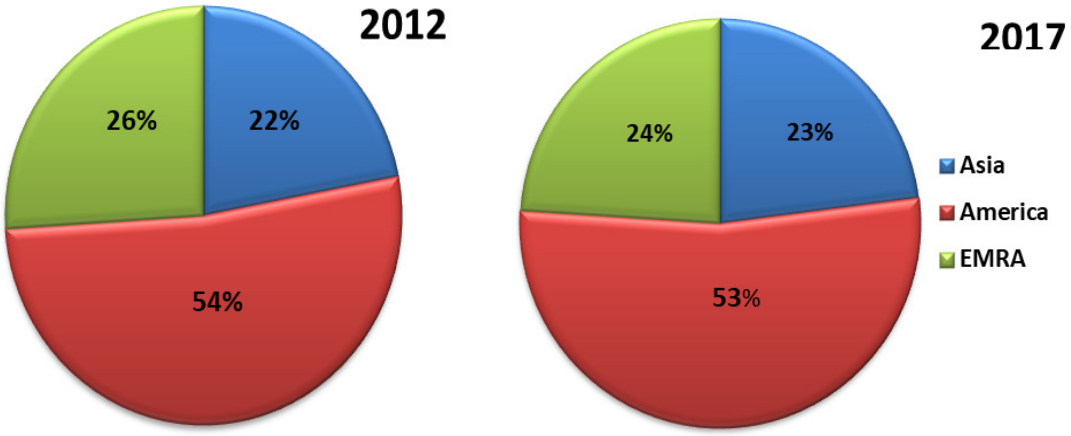


Figure 1.3: the graph shows figures of global market share for synthetic pesticides by region in 2012 and 2017.

There are two ways of applying of neonicotinoids on plants (Fig. 1.2).

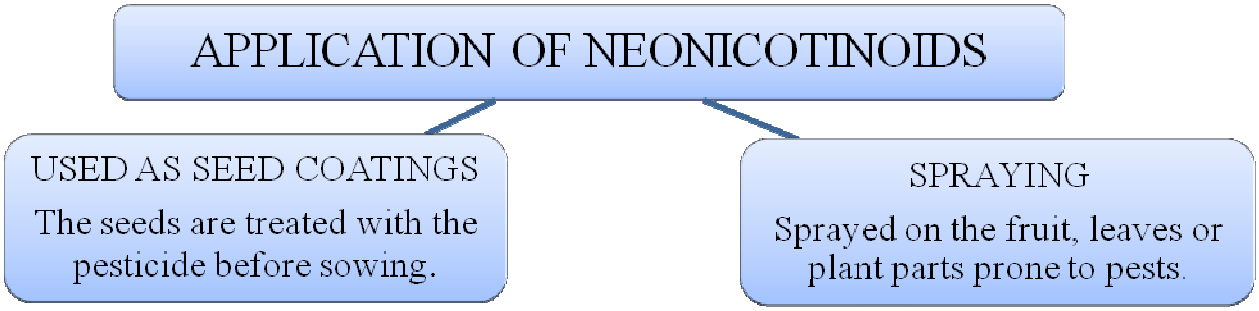
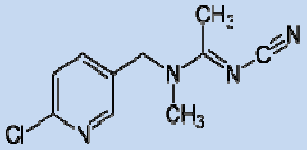
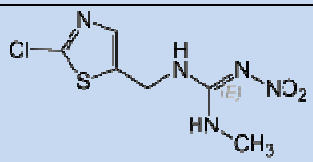
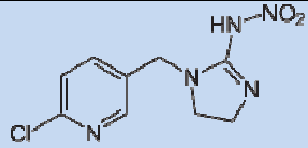
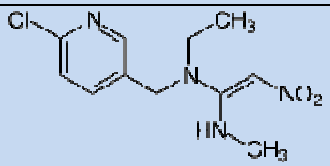
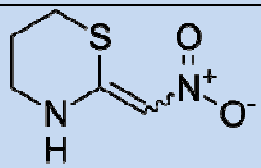
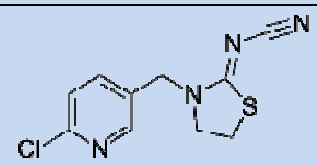
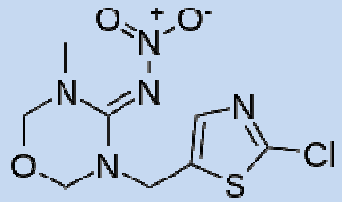

APPLICATION OF NEONICOTINOIDS
USED AS SEED COATINGS
The seeds are treated with the pesticide before sowing.
SPRAYING
Sprayed on the fruit, leaves or plant parts prone to pests.

Figure 1.3: Common ways of application of neonicotinoids in agricultural field

Their use is gradually increasing and is mainly being used for groundnut, corn, canola, soybean and majority of vegetables and fruits (Forero *et al*., 2017). Neonicotinoids are further classified into seven classes imidacloprid, acetamiprid, clothianidin, dinotefuran, nithiazine, thiocloprid and thiomethoxam (Table 1.1). Among these, imidacloprid, thiamethoxam and acetamiprid are the most commonly used.

Table 1.1: Types of neonicotinoids and their target pests

S.No	TYPES OF NEONICOTINOID	COMMERCIAL NAMES	MODE OF ACTION	TARGET PEST	STRUCTURE
1	Acetamiprid $C_{10}H_{11}CIN4$	Assail® Rescate® Saurus® Tristar®	They act as agonists on the polysynaptic nicotine acetylcholine receptors in the central nervous system of the pests.	Aphids, whitefies, bollworms	
2	Clothiandin $C_6H_8CIN_5O_2S$	Aloft® Belay insecticide® Clutch® Arena® Poncho® Prosper® Votivo®		NA	
3	Dinotefuran	Zylam® Transect® Safari® Venom insecticide®		NA	
4	Imidocloprid $C_9H_{10}CIN_5O_2$	Admire® Enforce® Mallet® Impulse Imidocloprid®		Termites, soil insects, fleas, Cane beetles, stink bugs and locusts	
5	Nitenpyram $C_{11}H_{15}CIN_4O_2$	Capstar®		Fleas and ticks	
6	Nithiazine $C_5H_8N_2O_2S$	Capstar®		Fleas and ticks	
7	Thiacloprid $C_{10}H_9CIN_4S$	Calypso® Biscaya®		Aphids and whiteflies	
8	Thiomethoxam $C_8H_{10}CIN_5O_3S$	Maxide® Endigo® Durivo® Agri-flex®, Actara®, Meridian®, Optigaurd®		Stem borers, Gall midge, leaf folder, and white backed planthoppers.	

Source: Pesticide information office/Fishel, Frederick M; Pesticide Toxicity Profiles

1.2.4. THIAMETHOXAM

Thiamethoxam is a neonicotinoid insecticide from thianicotinyl subclass used to control sucking and chewing insects in vegetables, ornamental, field crop, deciduous fruits, citrus, cotton and rice (Prabhakar *et al*., 2011). It is a broad spectrum pesticide, systemic with contact and stomach action. Its systemic activity has made it useful for treatment against foliar feeding insects through seed treatment or applying to trunks. It is an acetylcholine receptor (nAChR) agonist (Prabhakar *et al*., 2011).

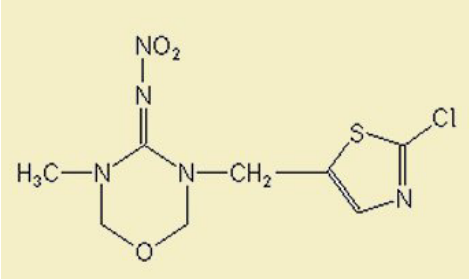


Figure 1.4: Structure of thiamethoxam

It is a crystalline odourless compound with a melting point of 139.1ºC. It has low molecular mass and a relative high water solubility of 4.1g/lt at 25ºC (Maienfisch *et al*, 2001).

REVIEW OF LITERATURE

The exhaustive use of chemical pesticides in the recent times to fight pests has indeed increased the agricultural yield but has been proved to be toxic and harmful to all kind of life forms in variety of ways. It is estimated by the World health organization (WHO) and the United Nation Environment Program (UNEP) that around 3 million workers and farmers associated with agriculture suffer from severe poisoning every year (Embrandiri *et al*., 2012). The extensive production of pesticide has taken a massive hike over the last decades which as discussed above are causing adverse health issues in individuals of all ages (George and Shukla, 2011). Chemical pesticide namely carbamate, pyrethoid, organophosphate and organochlorine are designated as potent carcinogens proving that they act as tumor initiators, promoters while some act as mutagens. They act as promoters by suppressing the immune system of the individual. Various kinds of cancer are associated with these pesticides such as breast cancer, pancreatic cancer, cervical cancer, prostate cancer, hemopoetic cancer. Also risk of leukemia, germ cell tumor in children, Non-Hodgkin's lymphoma has been highlighted in some studies (Parron *et al*., 2013).

2.1. NEONICOTINOID TOXICITY

Neonicotinoid was introduced in the market in 1990s. Some of the important characteristics of these pesticides include that their drug targets, being highly effective have low chance of resistance. This class of pesticide was considered to be to be harmless as compared to the traditional pesticide but gradually studies show that they cause toxicity in humans depending upon the level of exposure to the pesticide (Han *et al*., 2017). It also causes toxicity in plant by entering plant tissue and accumulating inside for a long time as they are easily translocated to different parts of the plant. Since, it is a relatively new class of chemical pesticide, therefore studies need to be conducted to study its toxicity in non-target organisms. Initial studies have shown lethal and sublethal effect of neonicotinoids on foraging behavior, learning and memory abilities of bees (Blacquiere *et al*., 2012).

Eight different studies have been reported by Cimino *et al*. in 2016 to investigate the effects of neonicotinoid exposure on human health. Four of these confirmed the association between chronic exposure of neonicotinoids and developmental and neurological toxicities. They

suggested that neonicotinoids have adverse effects in different species including mammals and also explained various pathways through which humans can be exposed to neonicotinoids.

Due to their high water solubility, neonicotinoids enter the plant and reside for a long time. For instance, acetamiprid and thiamethoxam were detected in the tissue of cherry leaves and fruits after 2 weeks of application. This is the main cause of probable environmental contamination which also affects the non-target organisms (Magalhaes *et al*., 2009).

There are many evidences which show that neonicotinoids cause reproductive toxicity prominently in rats and mice. Kapoor *et al*., 2011 reported patho-morphological changes in follicles, antral follicles and alretic follicles in female rats when treated with imidacloprid. They also observed decrease in ovarian weight and changes in the levels of LH, FSH and progesterone. Similarly, Bal *et al*. (2012) treated 24 male rats for 90 days with 3 doses of imidacloprid and observed decrease in the weight of epididymis, epididymal sperm concentration, vesicular seminalis, testosterone, gain in body weight and reduced glutathione values. An increase in lipid peroxidation, fatty acid concentration and high rates of abnormal sperm were also observed. Apotosis and fragmentation of seminal DNA was higher when treated with high doses. A study conducted on *in vitro* effects of acetamiprid and imidacloprid on mammalian reproduction by Gu *et al*. (2013) revealed no change in sperm motility and DNA integrity, but a mild effect on the fertilization ability.

When female BALB/c mice were treated with imidacloprid, immunosuppressive effect was seen by suppression of cell mediated immune response due to induction of histopathological changes in spleen and liver (Badgujar *et al*., 2013).They bind to the receptors just like acetylcholine but they are not broken down by the enzyme acetylcholine esterase which results in formation of an irreversible bond blocking the receptor which leads to nerve stimulation at even low concentration, paralysis in the long run and death at a very high concentration (Hirataa *et al*., 2017*).*

Role of imidacloprid was studied for excessive weight gain, its effects on obesity and symptoms of diabetes. The study was conducted to determine role of imidacloprid in lipid metabolism using 3T3-L1 adipocytes. It was determined that imidacloprid exposure resulted in accumulation of lipid and a significant elevation was seen in expression of an important regulator of adipocytes differentiation (Park *et al*., 2013).

When female mice were treated with imidacloprid for a period of 24 hours, mitotic inhibition and micronucleus formation was observed in mice (Kataria *et al*., 2015).A study conducted in Mexico established genotoxicity and cytotoxicity when human peripheral blood lymphocyte was exposed to 4 pesticides of class neonicotinoid at different concentration (Segura *et al*., 2011).

When a chloropyridylmethyl neonicotinoidnamed thiacloprid was administered in rats, an increase in micronucleus formation in BN cells was observed (Sekeroglu *et al*., 2011). A formulation of thiacloprid also caused an increase in the serum level of free triiodothyronine3 and free triiodothyronine4 in rats (Sekeroglu *et al*., 2013). In a study conducted in Turkey, potential genotoxicity of thiacloprid was studied on human peripheral blood lymphocytes. The study showed prominent increase in chromosomal aberrations, sister chromatid exchanges at all the different concentrations. Also there was an increase in the micronucleus at 75µg/mL for 24 hours and nucleoplasmic bridge formations at 150µg/mL for 48 hours treatment period (Kocaman *et al*., 2012). Another neonic pesticide, nitenpyramhave been reported to cause hematotoxicity in a study carried out in albino rat. The result showed that the total erythrocyte count (TEC) decreased 9% and 21% as compared to their controls (Khan *et al*., 2015).

Acetamiprid was administered to female wistar rats to study hematological toxicity through sub-acute oral exposure. It was reported that there was a significant reduction in levels of TEC, MCV and MHC but there was no decrease in levels of PCV, hemoglobin and MCHC (Mondal *et al*., 2009).

Studies have also shown that some neonicotinoids are involved in tumor formations. According to Environmental protection agency and World health organization, thiacloprid promote ovary tumors and uterine tumors in mice and rats respectively. Thiamethoxam produces liver tumors in female and male mice (Green *et al*., 2005). Similarly another pesticide Imidacloprid is said to increase the frequency of sister chromatid exchange and micronuclei formation in human peripheral blood lymphocytes (Segura *et al*., 2011).

Since neonicotinoid and nicotine are structurally similar and also share the same mode of action (Fig. 2.1). It has been well established through various studies that nicotine is directly related to initiation and growth of different types of cancer in humans (Kuroda *et al*., 2012). In a study conducted on HL-60 leukemia cells (Promyelocytic cell lines) by short term exposure of nicotine concentrations observed that it induced structural changes in chromatin resulting in activation of

AP-1 non neural cells by nicotine acetylcholine receptors dependent pathway which plays a major role in cell physiology and cancer proliferation (Landais *et al*., 2005).

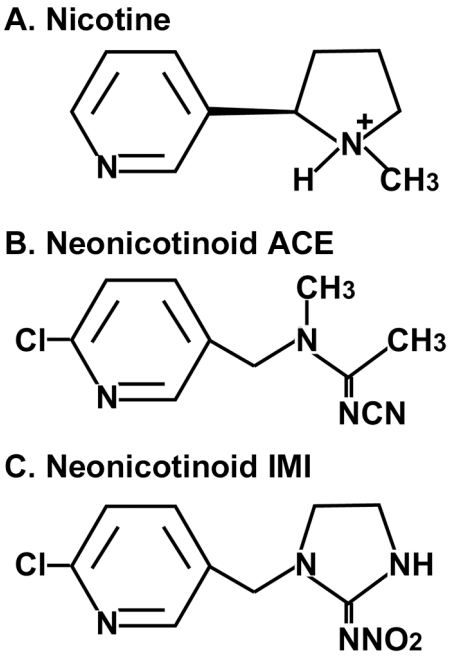


Figure 2.1: Comparison of structural similarities of neonicotinoids and nicotine
(Liu *et al* 2006)

2.2. HEPATOTOXICITY

Liver is a vital organ responsible for metabolism of drugs, toxins and their elimination. Therefore, it is the primary target organ for toxin encounter and damage by pesticides. There are many factors which contribute to hepatotoxicity, some of which are nutritional conditions, genetic factors and different treatments like medicines, pesticides and so on. The major evidences include abnormal enzyme levels and pathological and histological changes in liver. Some of the parameters used for evaluation of liver injury by chemicals include aspartate aminotransferase, alanine aminotransferase and various liver function tests (Malaguarnera *et al*., 2012).

In a study conducted on female albino rats, hepatotoxicity was studied by the assessment of histological changes and enzyme activity after imidacloprid exposure for 4 weeks. It was evident that treatment by $1/50^{th}$ dose resulted in dilation of central vein and sinusoids in between the hepatocytes. Also, levels of liver aspartate aminotransferase increased in higher doses. Significant increase was also seen in aspartate aminotransferase (AST), alkaline phosphatase (AKP) and alanine aminotransferase (ALT) plasma levels (Toor *et al*., 2013).

It is also reported that thiamethoxam produced severe toxicity in rabbit liver by induction of oxidative stress and alteration in antioxidants which lead to hepatic fibrosis that may progress to liver cirrhosis and cancer by production of pro-inflammatory cytokines and changes in the apoptotic signaling (Okle *et al*., 2017).

A study was conducted to observek the effect of imidacloprid on hepatotoxicity and nephrotoxicity in mice. It was observed that after 15 days of oral exposure, there was a significant increase in liver function test which is indicative of degenerative changes in the liver, at a dose much lower that the LD50 in mice (Arfat *et al*., 2014).

2.3 TRANSCRIPTOMICS

In the last decade, transcriptomics has evolved as an important approach for understanding of molecular genetics. This technology is used to study the transcriptome of an organism meaning the sum of its total RNA transcripts. It also plays an important role in identification of gene expression patterns and differential gene expressions simultaneously. Its further applications include identification of illness biomarkers and biological responses to stress and stimulations.

Analysis and understanding of altered gene expression lead to complex disorders like diabetes, heart diseases, various kinds of toxicities and cancer. One of its major applications implies evaluation of differential RNA expression which gives important insights of biological pathways and many molecular mechanisms regulating the fate, development and progression of diseases (Miura *et al*., 2017).

In 2014, Mesnage and his team conducted the transcriptome analysis for liver and kidney damage following chronic doses of roundup (glyphosate based herbicide) exposure in rats through drinking water. The liver and kidney were collected and transcriptomics microarray analysis was performed. The result clearly demonstrated up regulation and down regulation of many genes encoding for functional enzymes of liver and kidney. Disturbances were seen in the expression of 57 & 226 transcript clusters in liver and kidney. Akr1b1 and Ten1 were down regulated in liver and kidney respectively. Similarly up regulated transcripts in liver were small nucleolar RNAs. The mechanism involves interference in the production of aromatic amino acids tyrosine, tryptophan and phenylalanine in the Shikimate pathway. The main toxicity of GBH is due to the adjuvant present in the formulations like surfactants (Clair *et al*., 2012).
Some of the genes which were commonly expressed in kidney and liver were selected and validated by RT-qPCR (Table 2.1).

Table 2.1: Up and down regulation of commonly expressed genes in liver and kidney (Mesnage *et al*., 2015)**.**

S.No.	CLUSTERS	GENES	GENES ENCODING FOR	UP/DOWN REGULATION
1	1st 2 clusters	*Cpsf2, Cpsf3&Cpsf7*	Cleavage & Polyadenylation specific factors	All Up- Regulated
		*Hnrnp*l & *Hnrnpf*	Heterogeneous nuclear ribonucleoproteins	
		Sf3b5 & *Sf3a1*	Splicing factors	
		Luc7l3, Pnn, Prpf4b, Pnisr, Prpf39, Srek1,Ddx39b&Ddx39a.	RNA splicing	
2	2nd Clusters	*Men1, Setdb1, Suv420h2, Dot1l, Ehmt1, Ehmt2&Nsd1*	Chromatin modification family of enzymes	Up- regulated
3	3rd Clusters	*Idh3B* & *Idh3g*	Isocitrate dehydrogenase	Down- regulated
		Sdhc	Dehydrogenase	
		Sucla2 & *Suclg2*	Succinate CoA ligases	
		Atp5b & *Atp5d*	Mitochondrial F1 complex ATP synthases	

In another study sublethal effects of binary mixture of two neonicotinoids imidacloprid and thiacloprid in tissues of marine mussel were assessed. CDNA Microarray and quantitative PCR were carried out to evaluate gene expression in the digestive glands. It was followed by

proteasome profiling using 2-D electrophoresis of digestive glands cytosolic proteins (Dendero *et al*., 2010).

Thiamethoxam is a systemic insecticide which is used against broad range of agricultural and commercial pests and insects. Green and his co-workers (2005) carried out genotoxicity assessment of thiamethoxam including bacterial mutagenicity, cytogenetic, gene mutation, unscheduled DNA synthesis, and mouse micronucleus tests and observed that thiamethoxam was not genotoxic. However, it did cause an increased incidence of liver tumors in male and female Tif:MAGf mice when treated with the pesticide in the diet. It was also observed that there was a reduction in plasma cholesterol leading to cell death which in turn increased replication. This leads to development of tumors. In contrast, they found no increase in cancer incidences either in the liver, or at any other site, in rats fed on even higher thiamethoxam diets.

Therefore, to know the changes actually happening in the hepatic tissues followed by thiamethoxam exposure, it is important to know the hepatotoxicity of thiamethoxam in mice and observe the changes in gene expression in livers of mice exposed with the pesticide.

OBJECTIVES

1. To study the hepatotoxicity of thiamethoxam in mice
2. To observe the possible effect of thiamethoxam on gene expression in hepatic tissue.

METHODOLOGY

3.1. EXPERIMENTAL MODEL

50 male Swiss Albino mice of 20-25 days will be taken.

3.2. ANIMAL EXPERIMENTATION AND TREATMENT PLAN

1. 50 mice will be distributed in 5 groups (according to days of exposure) with each group exposed to three different concentrations.
2. The mice will be weighed and given chronic doses of pesticides orally in drinking water daily. The plan for the same is given in Table 3.1.
3. Weekly body weight will be taken for the entire duration of the study. After chronic exposure the mice will be weighed and sacrificed by cervical dislocation. Blood and Liver will be collected carefully and rinsed in cold saline, weighed and stored at -80ºC and analysed for various parameters (Table 3.2).

Table 3.1: Work Plan of pesticide treated male albino mice

GROUP	NO. OF DOSAGE	NO. OF MICE	AUTOPSY DAYS AFTER
I	$1/5^{th}$, $1/10^{th}$, $1/15^{th}$ of LD50	[3x 3] + 1= 10	1 month
II	$1/5^{th}$, $1/10^{th}$, $1/15^{th}$ of LD50	[3x 3] + 1= 10	3 month
III	$1/5^{th}$, $1/10^{th}$, $1/15^{th}$ of LD50	[3x 3] + 1= 10	6 month
IV	$1/5^{th}$, $1/10^{th}$, $1/15^{th}$ of LD50	[3x 3] + 1= 10	I year
V	$31/5^{th}$, $1/10^{th}$, $1/15^{th}$ of LD50	[3x 3] +1= 10	3 months (Recovery)

LD50 of thiamethoxam for mice is 871mg/kg body weight/ mice (Sinha *et al*., 2014).

Table 3.2: The biochemical assays to be performed for testing the hepatotoxicity of thiamethoxam in mice

S. No.	ORGAN /TISSUE /FLUIDS	BIOCHEMICAL OR OTHER PARAMETERS ASSAYED	REFERENCE
I	LIVER	**SERUM ENZYME TESTS:**	
		a. Ornithine carbamyl transferase (OCT)	Principles and methods of toxicology, by A. Wallace Hayes.
		b. Sorbitol dehydrogenase (SDH)	
		c. Lactate dehydrogenase (LDH)	
		d. Alkaline phosphatase	
		OTHER TESTS:	
		a. Reduced & oxidized glutathione (GSH-GSSG)	Hissin and Hilf *et al*., 1976.
		b. Reactive oxygen species (ROS)	Socci *et al*., 1999.
		c. GOT (Glutamate oxaloacetate Transaminase)	Enzopak Kits of Reckon Diagnostics
		d. GPT (Glutamate Pyruvate Transaminase)	
		e. Thiobarbituric acid reactive substances (TBARS)	Okhawa *et al*., 1979.
II	BLOOD	a. Glutathione	Jollow *et al*., 1974.
		b. Reactive oxygen species	Socci *et al*., 1999.
		c. GOT (Glutamate oxaloacetate Transaminase)	Enzopak Kits of Reckon Diagnostics
		d. GPT (Glutamate Pyruvate Transaminase)	
		e. Thiobarbituric acid reactive substances (TBARS)	Okhawa *et al*., 1979.
		f. Cholesterol	Accurex biological Kit
		g. iNOs	
		h. HCHO analysis	Swenson and Casida, 2013.

The metabolites produced in mice liver after thiamethoxam treatment will be analyzed by LC/MS.

3.3. TRANSCRIPTOME ANALYSIS

The gene expression study will be carried out by qPCR for various related genes in correspondence to the pesticide toxicity in mice model by SYBR green. The overview of the work plan is as follows:

1. Total RNA will be isolated from the liver tissues using Trisol.
2. Quantitation of total RNA will be carried out spectrophotometrically by noting down the ratio at A260/A280 to be ≥ 1.8.
3. This step will be followed by synthesis of cDNA using specific primers/universal primers by RT-PCR.
4. RNA expression will be evaluated by Q-PCR for specific mRNA involved in Apoptosis.
5. Housekeeping genes (GAPDH) will be taken as control
6. The genes under study are given in Table 3.3.

Table 3.3: Expression of selected genes under study

GENES (MICE)	ENCODING FOR PROTEIN	FUNCTION
Bcl2	B-cell lymphoma 2	• Apoptotic repressor • Regulates cell death by controlling the mitochondrial membrane permeability. • Abnormal expression of this gene is associated with cancer progression in tissues.
Casp9	Caspase-9	It is involved in activation cascade of caspases which in turn regulates execution of apoptosis. • It is activated when cytochrome C is released from the mitochondria. • It cleaves and activates caspase-3.

		• In mice, defect in this gene causes perinatal lethality
Casp3	Caspase-3	It s also involved in the activation cascade of caspases. • It functions by proteolytic cleavage of apoptotic target proteins. • In mice, defect in this gene causes reduced neuronal apoptosis which leads to hyperplasis, hearing loss, attenuated osyeogenic differentiation of bone marrow stem cells and post and pre-natal lethality
Bax	Apoptosis regulator BAX	• Triggers programmed cell death by binding to apoptotic repressor Bcl2. • Activates Caspase-3, leading to apoptosis • In stress condition, undergoes conformational change leads to release of cytochrome C and initiates apoptosis.
hmgcr	3-hydroxy-3 methyl glutaryl-Coenzyme A reductase	• It is involved in step III of the subpathway to synthesize (R) – mevalonate from acetyl-CoA. • It is a rate-limiting enzyme in cholesterol biosynthesis.

The above genes are selected to study the changes actually happening in the hepatic tissues followed by thiamethoxam exposure, it is important to know the hepatotoxicity of thiamethoxam in mice and observe the changes in gene expression in livers of mice exposed with the pesticide.

SIGNIFICANCE OF THE STUDY

The neonicotinoids are a new major class of highly potent insecticides that are used for crop protection and pest control. Increased caes of mouse liver tumors are common observations in chronic feeding studies with a wide range of agrochemicals. It is also true that the vast majorities of these chemicals are devoid of mutagenic activity and induce their effects by non-genotoxic modes of action. In some cases the modes of action are known and they give a clear indication of the likely human hazard; in others, data is incomplete. Similar is with the case of thiamethoxam which is a widely used pesticide now-a-days. Therefore, the current study aims at elucidating the effects of chronic doses of thiamethoxam on liver of Swiss albino mice and the changes in gene expression in the hepatic tissue. The findings of the proposed study may throw light on possible mechanism of hepatic carcinogenicity in the mammalian system.

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